

## PATENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION  
(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents  
 United States Patent and Trademark  
 Office  
 Box PCT  
 Washington, D.C.20231  
 ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 12 May 2000 (12.05.00)	Assistant Commissioner for Patents United States Patent and Trademark Office Box PCT Washington, D.C.20231 ETATS-UNIS D'AMERIQUE
International application No. PCT/US99/13340	Applicant's or agent's file reference 31737-PCT
International filing date (day/month/year) 11 June 1999 (11.06.99)	Priority date (day/month/year) 11 June 1998 (11.06.98)
Applicant HAYES, Mark, A. et al	

1. The designated Office is hereby notified of its election made:

in the demand filed with the International Preliminary Examining Authority on:  
11 January 2000 (11.01.00)

in a notice effecting later election filed with the International Bureau on:  
\_\_\_\_\_

2. The election  was  
 was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland  Facsimile No.: (41-22) 740.14.35	Authorized officer Juan Cruz  Telephone No.: (41-22) 338.83.38
---	---

**THIS PAGE BLANK (USPTO)**

## PATENT COOPERATION TREATY

## PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference <b>31737-PCT</b>	<b>FOR FURTHER ACTION</b> see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. <b>PCT/US 99/ 13340</b>	International filing date (day/month/year) <b>11/06/1999</b>	(Earliest) Priority Date (day/month/year) <b>11/06/1998</b>
Applicant <b>ARIZONA BOARD OF REGENTS et al.</b>		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

It is also accompanied by a copy of each prior art document cited in this report.

**1. Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

contained in the international application in written form.

filed together with the international application in computer readable form.

furnished subsequently to this Authority in written form.

furnished subsequently to this Authority in computer readable form.

the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2.  **Certain claims were found unsearchable (See Box I).**

3.  **Unity of Invention Is lacking (see Box II).**

4. With regard to the **title**,

the text is approved as submitted by the applicant.

the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

the text is approved as submitted by the applicant.

the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

as suggested by the applicant.

because the applicant failed to suggest a figure.

because this figure better characterizes the invention.

1

None of the figures.

**THIS PAGE BLANK (USPTO)**

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/13340

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 G01N27/447

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 200 050 A (IVORY CORNELIUS F ET AL) 6 April 1993 (1993-04-06) column 5, line 7 - column 6, line 68 column 13, line 7-21; figure 2 ---	1,2,6,8, 11,15
X	WO 96 27793 A (ALLTECH ASSOCIATES INC) 12 September 1996 (1996-09-12) page 15, line 31 - page 26, line 18; figures 3-6 ---	1-3,8, 10,11, 13,15,17
A	WO 96 04547 A (LOCKHEED MARTIN ENERGY SYS INC ;RAMSEY J MICHAEL (US)) 15 February 1996 (1996-02-15) page 39, line 31 - page 41, line 34 --- -/-	1-4,7-17

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

## \* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

20 August 1999

Date of mailing of the international search report

01/09/1999

Name and mailing address of the ISA  
European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Müller, T

**THIS PAGE BLANK (USPTO)**

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/13340

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MANZ A ET AL: "ELECTROOSMOTIC PUMPING AND ELECTROPHORETIC SEPARATIONS FOR MINIATURIZED CHEMICAL ANALYSIS SYSTEMS" JOURNAL OF MICROMECHANICS & MICROENGINEERING, vol. 4, no. 4, 1 December 1994 (1994-12-01), pages 257-265, XP000601273 ISSN: 0960-1317 ---	1-17
A	US 5 453 382 A (TSUDA TAKAO ET AL) 26 September 1995 (1995-09-26) cited in the application the whole document -----	1-17

**THIS PAGE BLANK (USPTO)**

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 99/13340

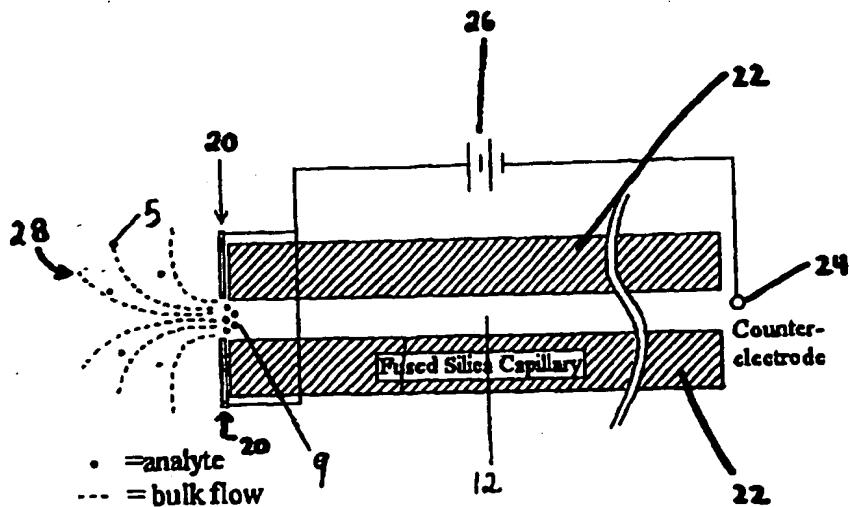
Patent document cited in search report		Publication date		Patent family member(s)		Publication date
US 5200050	A	06-04-1993	US	5298143 A		29-03-1994
WO 9627793	A	12-09-1996	AU	1547899 A		22-04-1999
			AU	5418296 A		23-09-1996
			CA	2187285 A		12-09-1996
			EP	0763199 A		19-03-1997
			JP	9511838 T		25-11-1997
			US	5759405 A		02-06-1998
WO 9604547	A	15-02-1996	AU	701348 B		28-01-1999
			AU	3150895 A		04-03-1996
			CA	2196429 A		15-02-1996
			CN	1168720 A		24-12-1997
			EP	0775306 A		28-05-1997
			JP	10507516 T		21-07-1998
			US	5858195 A		12-01-1999
US 5453382	A	26-09-1995		NONE		

**THIS PAGE BLANK (USPTO)**

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : <b>G01N 27/447</b>		A1	(11) International Publication Number: <b>WO 99/64851</b>
			(43) International Publication Date: 16 December 1999 (16.12.99)
(21) International Application Number: PCT/US99/13340		(81) Designated States: CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).	
(22) International Filing Date: 11 June 1999 (11.06.99)			
(30) Priority Data: 60/088,956 11 June 1998 (11.06.98) US		Published <i>With international search report.</i>	
(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application US Filed on 60/088,956 (CIP) 11 June 1998 (11.06.98)			
(71) Applicant (for all designated States except US): ARIZONA BOARD OF REGENTS [US/US]; Arizona State University, Box 873211, Tempe, AZ 85287 (US).			
(72) Inventors; and			
(75) Inventors/Applicants (for US only): HAYES, Mark, A. [US/US]; 3583 W. Barcelona Drive, Chandler, AZ 85226 (US). POLSON, Nolan, A. [US/US]; 1300 W. Shannon Street, Chandler, AZ 85224 (US).			
(74) Agents: SORELL, Louis, S. et al.; Baker & Botts, LLP, 30 Rockefeller Plaza, New York, NY 10112-0228 (US).			

## (54) Title: CONTROL OF FLOW AND MATERIALS FOR MICRO DEVICES



## (57) Abstract

The present invention generally relates to methods and devices for the control of the movement of fluids and electrically charged sample components within those fluids. More particularly, the present invention permits exclusion or concentration of specifically chosen sample components within a fluid. The present invention provides an analytical device, either microchip- or capillary-based, having the means to exclude specific sample components of interest from a capillary or channel for the purpose of preconcentration or control of movement of sample components. Such a control system includes a means for controlling the flow of the fluid in the channel and the placement of an electrode at the immediate entrance of each channel on such devices so that material may be directly manipulated by effects of both bulk flow and electrically driven migration.

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

CONTROL OF FLOW AND MATERIALS FOR  
MICRO DEVICES

INTRODUCTION

5 The present invention generally relates to methods and devices for the control of the movement of fluids and electrically charged sample components within those fluids. More particularly, the present invention permits exclusion or concentration of specifically chosen sample components within a fluid.

10 The present invention provides an analytical device, either microchip- or capillary-based, having the means to exclude specific sample components of interest from a capillary or channel for the purpose of preconcentration or control of movement of sample components. Such a control system includes a means for controlling the flow of the fluid in the channel and the placement of an electrode at the immediate entrance of each channel on such devices so that material may be directly manipulated by either or both of the effects of both bulk flow and electrically driven migration.

15 BACKGROUND OF THE INVENTION  
Capillary zone electrophoresis (CZE) is an efficient analytical separation technique which utilizes differences in mobility of sample components in an electric field based on the electrical charge and molecular size and shape of the 20 sample component. Conventional CZE systems typically comprise a buffer-filled capillary with outlet and inlet ends disposed in two reservoirs into which one sample is injected, a means for applying voltage to the capillary resulting in migration of the sample through the capillary, and a means for detecting the sample zone.

25 Sample injection systems and capillary zone electrophoresis channel systems have been integrated together on planar glass substrates for separation of sample components as described by Harrison et al. (1992, Anal. Chem. 64:1926-1932) and Seiler et al. (1993, Anal. Chem. 65:1481-1488). Additionally, capillary electrophoresis on microchips has been described by Manz et al., (1992, J. of

Chromatography 593:253-258). Total chemical analysis systems (TAS) in which sample transport, chromatography or electrophoretic separations and detection are all performed have also been developed.

One of the limitations of conventional CZE is the extremely small amount of sample which must be used in order to obtain separation or resolution of sample components. The use of small volume samples results in low amount of sample components of interest representing a major limitation in the detectability of sample components. On the other hand, the larger the sample volume introduced into the capillary, the broader the sample component peaks will be. Attempts to increase injection sample volume typically leads to a breakdown in resolution due to broadening of the peaks attributable to individual sample components which one is actually trying to resolve or separate and possibly leads to generation of laminar flow inside the capillary.

A number of techniques have been developed for increasing the concentration of specific sample components of interest and narrowing the width of the injected sample. One such technique involves the use of a solid-phase adsorption medium followed by a sequential combination of pressure- and electrically-driven flows as described in United States Patent No. 5,453,382. Using such a technique, the solution containing the sample component of interest is applied to the solid phase adsorption medium under conditions which permit sorption of the sample component of interest to the adsorption medium. The environment of the medium is then altered to promote desorption of the concentrated sample component and a voltage gradient is induced across the medium to induce electroosmosis. United States Patent No. 5,340,452 also describes a similar method for increasing the concentration of sample components prior to electrophoresis by using an active material which selectively retains the sample components of interest at the inlet end of the capillary tube.

For some specialized samples, another obstacle to successful separation of components of a solution results from the low strength of the electric field in the buffer bordering the sample solution and the column buffer. To circumvent this problem, water or diluted buffer may be removed from the capillary or column using electro-osmotic flow while the sample components are stacked in a

support buffer thereby concentrating the sample components in a sample with a minimum amount of laminar flow. Such a method is described in United States Patent No. 5,116,471.

For large volume samples in constrained containers, pressurized flow and countermigration can be used to increase the overall concentration as described by Hori et al. (1993, *Anal. Chem.* 65:2882-2886). The sample is introduced into a first vessel containing buffer which is connected to another vessel by a glass tube. An electrode extending into the first vessel applies a voltage to the sample while suction pressure is applied. The sample concentration increases throughout the first vessel rather than concentrating the sample in a discrete portion of that vessel because the applied potential field is unconstrained throughout the buffer volume. Because the concentration increase and electric fields are dispersed throughout the entire first vessel volume this technique is not applicable as a small volume injection/preconcentration technique. Moreover, this arrangement does not allow for micromanipulations such as electrophoretic separation within the vessel containing the concentrated sample.

Hence, none of the aforescribed methods provide for concentration of sample components upon immediate introduction into a constrained small volume flow path which receives a fluid sample without the use of complicated systems such as discontinuous buffer systems and, in some instances, microengineered absorption devices. Accordingly, there exists a need in the art for more precise and efficient methods and devices for increasing the concentration of sample components of interest within a fluid sample while maintaining a consistent buffer and without microengineering absorption systems.

25

#### SUMMARY OF THE INVENTION

Accordingly, it is an object of the present invention to provide a novel, more efficient method for controlling the movement of fluids and electrically charged species, referred to as sample components, within those fluids which permits exclusion or concentration of specifically chosen species within a constrained fluid-flow path.

It is another object of the invention to provide an analytical electrophoretic arrangement including microchips or capillaries which excludes specific sample components of interest from a capillary or channel for the purposes of preconcentration or control of movement of materials.

5 It is a further object of the present invention to provide an arrangement in which preconcentration and manipulation is achieved within a single constrained flow pathway system. More particularly, the sample is preconcentrated in a portion of the constrained flow pathway and is manipulated as it travels through the pathway.

These and other objects of the invention are obtained by a method for  
10 controlling the movement of a specific sample component in a fluid sample comprising:

- (a) providing a constrained fluid pathway having an inlet;
- (b) introducing the fluid sample into the inlet of the constrained fluid pathway;
- 15 (c) providing an electrode mounted at the inlet of the fluid pathway, the electrode being entirely external to the constrained fluid pathway;
- (d) applying voltage to the electrode to create a voltage gradient within the constrained fluid pathway to promote electrophoretic migration of the sample component; and
- 20 (e) adjusting the flow rate of the fluid approximately equal to and opposite to the electrophoretic migration of the sample.
- (f) adjusting the electrophoretic migration rate to be approximately equal and opposite to the flow rate of the fluid.

25 wherein movement of the specific sample component ceases.

The invention further provides an electrophoretic apparatus for controlling the movement of an sample component in a fluid sample comprising:

- (a) at least one constrained fluid pathway having an inlet and an electrode mounted at the inlet of the constrained fluid pathway and entirely external to the constrained fluid pathway; and
- 30 (b) a power supply for supplying a voltage to the electrode.

It is another object of the invention to provide an electrophoretic apparatus for controlling the movement of an sample component in a fluid sample comprising:

- 5 (a) at least one injection fluid pathway having an electrode mounted at the inlet of said the pathway;
- (b) at least one separation fluid pathway having an electrode mounted at the inlet of said pathway;
- (c) at least one power supply for providing voltage between the electrodes; and
- 10 (d) means for regulating the bulk flow within the channels.

The present invention can be utilized in methods and devices for manipulating, testing, probing, or analyzing sample fluids of any kind where fluid manipulations are utilized for preconcentration, chemical reaction, injection, detection, or movement, or cessation of movement, of components of interest in a 15 sample fluid.

In one embodiment, the present invention is directed to an analytical device having a plurality of channels with electrodes placed at the immediate entrance of all or selected channels and a method for regulating the bulk flow within the channels. When the bulk flow is set approximately equal to and opposite the 20 electrophoretic migration of specific sample components of interest, the movement of those specific sample components ceases. The introduction of an electric field between the electrodes within the channel, coupled with control of bulk flow, allows selected sample components of interest to be excluded or preconcentrated immediately upon introduction of the fluid sample into the channel.

25

#### BRIEF DESCRIPTION OF THE DRAWINGS

Further objects and advantages of the invention will be apparent from a reading of the following description in conjunction with the accompanying drawings, in which:

30 Figure 1 is a schematic drawing of a fused silica capillary arrangement with electrodes placed immediately at the inlet to provide the voltage control within

the capillary in accordance with the invention;

Figures 2(a), 2(b) and 2(c) are schematic drawings of a micro-device apparatus having an injection channel and a separation channel in accordance with the invention;

5                   Figure 3 is a schematic drawing of a micro-device apparatus indicating the preconcentration of materials at the immediate entrance to a channel where the voltage within the buffer reservoir is held constant in accordance with the invention;

10                  Figure 4 is a schematic drawing of the theoretical profile of the preconcentration of material at the immediate entrance to a capillary showing the concentration of desired materials;

Figure 5 is a graph showing the normalized fluorescence intensity versus distance outside the capillary entrance for two control experiments;

Figure 6 is a graph showing the normalized fluorescence intensity versus number of pixels (1 pixel = 0.24  $\mu\text{m}$ ) outside a capillary entrance ;and

15                  Figures 7(a) and (b) are fluorescence micrographs of a capillary entrance before and after, respectively, preconcentration of 200 nm fluorescently labeled latex micro spheres for 270 seconds.

#### DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides novel methods and devices for exclusion or concentration of specifically chosen sample components within fluids through the control of fluid movement and electrophoretic migration of charged sample components within those fluids. Typically the fluid sample is delivered or injected into a restricted flow path such as a channel or capillary. For purposes of the present invention, the flow path is preferably less than 200 microns in diameter.

25                  Precise control of fluid manipulation, sample component movement and solution injection systems are accomplished by carefully controlling the voltage field gradients and the bulk flow within each channel on a micro-device.

The principle of electrophoretic focusing as a means of sample component exclusion from a capillary or channel can be applied to the microscale analytical device described herein. The apparatus and processes disclosed herein may

be used on microchip instrumentation in conjunction with control fluid dynamics in channels formed into or onto semiconductor devices. As used herein, the term "microchip" includes a semiconductor device comprising silica or any other substrate which may be used in microfluidic devices, which may be used in or in conjunction with a computer.

5 The present invention also provides for the placement of an electrode at the immediate entrance of each channel on a micro-device so that material movement may be directly manipulated by electrically-driven migration, i.e., electrophoretic migration. The present invention also provides control of bulk flow 10 of the fluid within the channel. Bulk flow may be positive or negative depending upon the magnitude and direction of electrically-driven flow, i.e., electroosmosis, or various other sources of flow such as pressure, convection, capillarity, etc. Voltage gradients may likewise be manipulated to provide electrophoretic migration in either direction.

15 The introduction of an electric field resulting in electrophoretic migration of a specific sample component, coupled with manipulation of bulk flow equal and opposite to electrophoretic migration, results in cessation of movement of those specific sample components. Thus, the independent control of these parameters provides for absolute control of movement of sample components within the fluid 20 about a micro-device.

25 The method of the invention comprises as a first step, the introduction of a sample containing the sample component of interest into a channel or capillary that has been filled with buffer. Sample introduction may be accomplished using a syringe by which the sample solution is injected into the channel. Alternatively, the introduction of the sample can be performed according to standard procedures, including but not limited to the use of electroosmotic flow, electro-kinetic pumping, or pneumatic pumping.

An electrophoretic arrangement in which a capillary is utilized to create the restricted flow path is shown in Figure 1. In this arrangement electrodes 20 are located external to and mounted onto a fused silica capillary. A counter electrode 30 is placed at a location remote from electrodes 20 and forms a circuit therewith. A 24

high voltage is applied to the electrodes 20 and 24 by power supply 26. A reservoir 28 including buffer bulk flow materials is in fluid contact with the capillary. A sample 5 including charged components is introduced into the reservoir and moves towards the entrance 9 of the capillary in the presence of the applied voltage which 5 induces electrophoretic migration. Thus, the charged components in analyte 5 are concentrated at the entrance 9 of the capillary 22.

The present invention also provides a micro-analytical separation device comprised of etched or molded channels whereby various channels are used for separation and analysis purposes and others are distinctly used for the purpose of 10 injection or material movement illustrated in Figs. 2(a-c). As shown in Figure 2a the system includes an injection channel 2 and separation channel 4. Sample material is injected to fill the injection channel 2 in between the separation channels 4 as depicted in Figure 2b. To prevent unintentional introduction of material movement, commonly referred to as trailing or leaking, into the main separation channel after injection 15 ceases, a small voltage is applied to the two injection channel electrodes 5. As illustrated in Figure 2c, after the initial injection the electrodes are used to create an appropriate voltage gradient to prevent unwanted introduction of materials into the separation channel thereby concentrating desired components in separation channel 4. By manipulating flow and the voltage fields independently, positive, negative and 20 neutral molecules may be manipulated as a group or individually.

A high voltage is applied by power supply means between the inlet and outlet end of the channel or capillary through electrode means. The voltage used is not critical to the invention and may vary widely depending on the sample component(s) to be excluded or concentrated. Conditions for selecting appropriate 25 voltage conditions will depend on the physical properties of the sample component(s) and can be determined by those of skill in the art.

To preconcentrate sample components of either positive or negative charge, the method of the invention further comprises setting the bulk flow in the channel or capillary approximately equal to and opposite to the electrophoretic 30 migration rate of the material. The bulk flow in the capillary may be generated and controlled by either electroosmosis, pressure or various other mechanisms. Bulk flow

may be created and controlled by electroosmotic pumping devices, pneumatic devices, or directly by electroosmosis with dynamic control and monitoring. Thus the sample component of interest is drawn toward the channel by bulk flow, but is excluded from the channel by the voltage field effects on a narrow range of materials 5 with similar electrophoretic migration rates thereby excluding or concentrating the sample component of interest at the immediate entrance of the capillary or channel.

Alternatively, any constrained fluid pathway, for example a fused silica or teflon capillary, where separation or injection of materials of interest are performed may be included in the device. Each channel or continuous fluid pathway where 10 control of material movement is desired is constructed with an electrode adjoining the entrance and exit of the channel or pathway. Electrodes are placed at the entrance of the side channels to control the voltage field allowing electrophoretic migration to occur, and electroosmosis if the source of flow in the particular channel. In this manner the invention provides for integration of preconcentration and analysis within 15 the constrained fluid pathway.

In the preferred embodiment of the invention as shown in Figure 2a-c, the injection channel 2 is perpendicular to the separation channel 4, although the geometry of this intersection is not of direct importance to the concepts presented here. Electrodes 5, 6 are located at the immediate entrances of channels 5, 6 and are 20 electrically connected to the junction where the two channels 2, 4 intersect.

Placement of an electrode at the immediate entrance of a capillary or channel and at the junction with another channel or buffer reservoir, creates a chemical voltage gate, in that movement of materials may be independently controlled by simply varying the voltage field gradient and the flow rate within the particular channel. At this chemical 25 voltage gate, materials of interest may be totally excluded from entering the adjoining channel or selectively permitted to enter the channel by using electrophoretic focusing techniques.

In another embodiment of the invention shown in Figure 3 a reservoir containing a buffer solution 5 is placed in fluid contact with a channel 12 and an 30 electrode 9 is placed at the immediate entrance to that channel 11. The buffer reservoir is maintained at the same voltage as the entrance electrode, thus the material

will not undergo electrophoretic migration within the reservoir. However, the charged materials will move toward the channel entrance at the same rate as the bulk flow. At the immediate entrance of the channel the effects of the applied voltage field influences the charged materials, thus inducing electrophoretic migration. Since the 5 bulk flow within the channel is approximately equal to and opposite the electrophoretic migration, the charged material of interest stops.

The flow rate of fluids may be controlled by, for example, the following techniques: pressure induced flow, capillary, and electroosmosis as taught by Giddings (1991, *Unified Separations Science*, Wiley-Interscience, New York, 10 Chapt. 3). More specifically, pressure can be controlled by any physical or chemical means which will generate controllable flow or pressure. Capillarity can be controlled via chemical, electrochemical or photo-induced surface or solution changes as taught by Gallardo et al. (1999, *Science* 283:57-60). Electroosmosis can be controlled by external radial electrostatic fields as taught by Tsuda (1998, *Handbook of Capillary 15 Electrophoresis*, Ed. J.P. Landers, 2<sup>nd</sup> ed., CRC Press, Boca Raton, Chap. 22).

The methods and devices of the present invention may be used for purposes of manipulating, testing, probing, or analyzing fluids of any kind where fluid manipulations may be used for preconcentration, chemical reaction, injection, detection, or movement or restriction of movement, of the materials of interest. The 20 manipulations provided for by the methods and devices described herein will allow for precise liquid injection and handling within a micro-chemical analysis device in addition to the ability to increase local concentration of materials by several orders of magnitude.

Preparation of specific embodiments in accordance with the present 25 invention will now be described in further detail. These examples are intended to be illustrative and the invention is not limited to the specific materials and methods set forth in these embodiments.

The examples discussed hereinafter were conducted using the following standard chemicals and instrumentation, unless otherwise stated:

30 *Chemicals and Materials.* Sodium dihydrogen phosphate and anhydrous ethyl alcohol (Aldrich Chemical Company, Milwaukee, WI); and

phosphoric acid (EMG/NCV Science, Gibbstown, NEW JERSEY) were used as received. Capillaries were 45 cm in length (150  $\mu$ m o.d. - 20  $\mu$ m i.d.) fused silica and were purchased from Polymicro Technologies (Phoenix, AZ). 0.2  $\mu$ m carboxylate modified yellow-green fluorescent (505/515) latex micro spheres were purchased from Molecular Probes (Eugene, OR). The capillary electrophoresis buffer used for the latex micro sphere experiments was 100 mM phosphate buffer, adjusted with phosphoric acid to pH 5.1.

Instrumentation. The capillary electrophoresis system was built and used a CZE1000R high voltage power supply from Spellman High Voltage Electronics Corporation (Hauppauge, New York). The vacuum pump system was purchased from Cenco Hyvac (Fort Wayne, IN). The laser source was a 442/325 nm 100 MPA: (Omnichrome Laser, Chino, Cat Scan). Image viewing was accomplished with a case closed-5E CCD camera (HutchNet, East Hartford, Construction) integrated to an Olympus Vanex stereo microscope (Tokyo, Japan). Data collection and analysis were accomplished using Labview software and an Imaq Pci- 1408 image acquisition board by in-house program development (National Instruments, Austin, TX). Data analysis was also performed on Microsoft Excel spreadsheet program using an Optiplex GXI Pentium 233 (Dell Computer Corporation, Round Rock, TX). The fluorescent signal was monitored from the carboxylate modified latex micro spheres as vacuum and voltage fields were adjusted.

#### Example 1

Experiments were performed to effectively demonstrate the increased local concentration of specific materials using a capillary 30 and reservoir 32 arrangement shown in Fig. 4. The tip of the capillary was coated with metal 34 thereby providing a metal electrode. These experiments were performed with fluorescence microscopy, fluorescently labeled latex microspheres, vacuum flow and a metal-coated capillary tip.

The presence or location of carboxylate-modified latex spheres were directly observed with the microscope under the effects of vacuum induced flow. The voltage was then empirically adjusted until the micro spheres were excluded from entering the capillary due to the electrophoretic migration rate of the micro spheres.

The intensity of the fluorescent signal which is directly related to concentration was monitored. Only a selected probe area, of approximately 2.5  $\mu\text{m}$  x 120  $\mu\text{m}$  parallel, and centered with the bore of the capillary immediately outside the entrance was quantitated for the fluorescence intensity changes.

5 First, control experiments were performed to determine if adsorption or other unknown processes were responsible for the increased fluorescence. These control experiments consisted of using either the voltage field only (- 14 kV) or the vacuum-induced flow only 1.2 in Hg across a 45 cm long, 20  $\mu\text{m}$  i.d. capillary. As illustrated in Figure. 5 the fluorescent signal was monitored and quantitated for 4  
10 minutes. The fluorescent signal was normalized with the fluorescent signal obtained at  $t = 0$  minutes to eliminate any existing background fluorescence from the temporal data. The normalized fluorescent signal of the control experiments remained at a value of  $1.75 \pm 9.32$  ( $n = 11$ ) throughout the 4 minutes of the experiment (Figure 6). No  
15 increase in fluorescent intensity was observed over the experimental period indicating that no unknown mechanisms nor adsorption to the capillary tip and walls contributed to the increased fluorescent intensity in the following experiments.

Experiments were performed to demonstrate preconcentration once the electrophoretic migration rate within the channel in the capillary was adjusted to be equal to and opposite the bulk buffer low rate. As with the control experiments, the  
20 fluorescence intensity was normalized and then monitored for 4 minutes ( $n = 4$ ). The voltage empirically determined to generate an electrophoretic migration rate which counterbalanced the bulk flow rate was 14kV. Figures 7(a) and (b) are fluorescence micrographs of a capillary entrance before and after, respectively, preconcentration of 200 nm fluorescently labeled latex micro spheres for 270 seconds. As illustrated in  
25 Figure 7b and Figure 4 the largest fluorescence intensity changes occurred within 33  $\mu\text{m}$  of the capillary entrance. Due to dynamic range limitations, the fluorescent intensity at the entrance to the capillary saturated the CCD and therefore quantitation of this effect must be performed some 19.2  $\mu\text{m}$  outside the entrance to the capillary. The normalized fluorescent signal at 19.2  $\mu\text{m}$  resulted in an increase in fluorescence  
30 intensity approximated by a linear equation ( $y = mx + b$ ) where  $m$  is 0.042 arb. units/min and  $b$  is 0.99 arb. units ( $R^2 = 0.938$ ,  $P \leq 0.01$ ). The initial concentration of

the micro-spheres was  $1.473 \times 10^{10}$  micro spheres/mL.

5 The preconcentration build-up over time can be modeled as the formation as an exponential zone superimposed on a background of constant solute concentration for materials accumulating up behind a partially rejecting barrier such a filter. The filter in this case is the exclusion of the micro spheres from the capillary by the applied voltage field and the resulting electrophoretic migration rate. Assuming the system will reach steady state conditions after a given time, the background concentration of the micro spheres is equal to  $J_a/v$ , initial flux over velocity. The concentration build-up of the micro spheres is given by the following equation:

10 
$$c = J_a v + (c_o - J_a l v) \exp(-lv/y/D_f)$$

where the concentration of the micro spheres is given by  $c$ , the flux of the micro spheres is given by  $J_a$ , the velocity of the flow towards the barrier is  $v$ , the original concentration of micro spheres is given by  $c_o$ , the distance from the barrier is given by  $y$ , and the total diffusion of the micro spheres is given by  $D_f$ . A plot of concentration of desired components versus location in the arrangement is shown in Figure 4.

15 Although the present invention has been described with reference to latex micro spheres and fused silica capillaries providing the constrained fluid pathway, it should be understood that various modifications and variations can be easily made by those skilled in the art without departing from the spirit of the invention. Such modifications are intended to fall within the scope of the claims. Accordingly, the foregoing disclosure should be interpreted as illustrative only and not in a limiting sense. Various publications are cited herein, the contents of which are incorporated, by reference, in their entireties.

CLAIMS

1. A method for controlling the movement of a specific sample component in a fluid sample comprising:
  - (a) providing a constrained fluid pathway having an inlet;
  - (b) introducing the fluid sample into the inlet of the constrained fluid pathway;
  - (c) providing an electrode mounted at the inlet of the fluid pathway, the electrode being entirely external to the constrained fluid pathway;
  - 10 (d) applying voltage to the electrode to create a voltage gradient within the constrained fluid pathway to promote electrophoretic migration of the sample component; and
  - (e) adjusting the flow rate of the fluid approximately equal to and opposite to the electrophoretic migration;
- 15 wherein movement of the specific sample component ceases.
2. The method of claim 1 wherein the constrained fluid pathway is a channel.
3. The method of claim 1 wherein the constrained fluid pathway is a capillary tube.
4. The method of claim 1 wherein the constrained fluid pathway is less than 200 microns in diameter.
- 20 5. The method of claim 1 wherein the flow rate of the fluid sample is controlled by electroosmosis.
6. The method of claim 1 wherein the flow rate of the fluid sample is controlled by pressure.

7. The method of claim 1 wherein the constrained fluid pathway is a channel on a microchip.
8. An electrophoretic apparatus for controlling the movement of an sample component in a fluid sample comprising:
  - 5 (a) at least one constrained fluid pathway having an inlet and an electrode mounted at the inlet of the constrained fluid pathway and entirely external to the constrained fluid pathway; and
  - (b) a power supply for supplying a voltage to the electrode.
- 10- 9. The apparatus of claim 8 wherein the constrained fluid pathway is a channel located on a microchip.
10. The apparatus of claim 8 wherein the constrained fluid pathway is a capillary.
11. The apparatus of claim 8 further comprising a buffer reservoir for containing a buffer solution in fluid contact with the constrained fluid pathway.
- 15 12. The apparatus of claim 8 wherein the constrained fluid pathway is a channel in a microchip.
13. The apparatus of claim 8 wherein the constrained fluid pathway is a capillary.
14. The apparatus of claim 8 wherein the diameter of the constrained fluid pathway is less than 200 microns in diameter.
- 20 15. An electrophoretic apparatus for controlling the movement of an sample component in a fluid sample comprising:
  - (a) at least one injection fluid pathway having an electrode mounted at the inlet of said the pathway;

- (b) at least one separation or further fluid transfer fluid pathway having an electrode mounted at the inlet of said pathway;
- (c) at least one power supply for providing voltage to the electrodes; and
- 5 (d) means for regulating the bulk flow within the channels.

- 16. The method of claim 15 whercin the constrained fluid pathway is a channel in a microchip.
- 17. The method of claim 15 whercin the constrained fluid pathway is a capillary.